

protofilaments. Previous experiments to characterize the stiffness of microtubules have generally relied on heterogeneous ensembles of microtubules with different structures, complicating the interpretation and comparison of stiffness measurements. We report on a kinesin-driven microtubule gliding experiment that simultaneously determines the stiffness and protofilament number (diameter) of individual microtubules, allowing us to distinguish stiffness for microtubules of 12-14 protofilaments (including two different 14 protofilament lattice structures). The scaling of our stiffness measurements are consistent with previous theories connecting protofilament number to stiffness, but also suggest an alternate correlation between stiffness and protofilament superhelical pitch.

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A Structure-Function Study of Map Tau: Analyzing Distinct Map Tau Domains in Mediating Microtubule Assembly and Bundling using Synchrotron SAXS

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The microtubule-associated protein tau (MAP tau) promotes the assembly of tubulin into microtubules, a major component of the eukaryotic cytoskeleton. In neurons, MAP tau is especially abundant in the axon and plays a critical role in axonogenesis [1]. However, aberrant tau function has been implicated in many neurodegenerative diseases, such as Alzheimer's and supranuclear palsy [2,3]. Thus, there is a clear need to understand the structure of MAP tau with regards to its interactions with microtubules and its role in neuropathies. The six isoforms of MAP tau are often described as sequentially having a N-terminal (consisting of a projection domain and a proline-rich region), microtubule-binding repeats, and a C-terminal. While the function of the microtubule-binding repeats is well understood, the N- and C- terminals have been particularly difficult to characterize structurally and functionally due to the presence of intrinsically disordered domains. Using synchrotron small-angle X-ray scattering (SAXS), we examined the higher-order assembly of microtubules induced by varying concentrations of wild-type MAP tau under cell-free solution conditions. The functional dependence of the projection-domain, proline-rich region, and C-terminal were also examined by utilizing distinct constructs resulting in MAP tau with deletion domains. Not only do these results correspond well to axonal microtubule-tau bundles in vivo, we find that the modulation of the bundling of MAP tau is isoform-dependent. Surprisingly, the N-terminal tail of MAP tau is not necessary for higher-order assembly, as some models have suggested [4].

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Stu2p, the Budding Yeast Homologue of XMAP215, is a Weak Microtubule Polymerase that Promotes Rescues

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Stu2p is a member of the Dis1/XMAP215 family of polymerases and is required for spindle orientation and metaphase chromosome alignment, and for anaphase B spindle elongation. Despite Stu2p having a phenotype suggesting that it promotes microtubule growth, studies till now with purified proteins indicate only that Stu2p antagonizes growth. To investigate the effect of Stu2p on microtubule dynamics in vitro, we purified recombinant Stu2p from insect cells and studied its effect in a total-internal-reflection fluorescence (TIRF) microscopy assay: GMPCPP-stabilized microtubules (seeds) were immobilized on the glass surface and varying amounts of fluorescently labeled porcine-brain tubulin, GTP and Stu2p proteins were added to the chamber. Images were acquired using time-lapse microscopy. In the presence of tubulin, Stu2p increased the microtubule growth rate, though to a lesser extent than XMAP215. Stu2p had no effect on the catastrophe frequency, but significantly increased the rescue frequency. In conclusion, Stu2p shares functional properties with its metazoan homolog XMAP215, though its polymerase activity is weaker. These properties go some way to explaining the phenotypes of the Stu2p-depleted cells.

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Cooperative Interactions in the Microtubule-Severing AAA ATPase Spastin

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Spastin is a hexameric ring AAA ATPase that severs microtubules. To see how the ring complex funnels the energy of one or multiple ATP hydrolysis events to the site of mechanical action, we investigate here the cooperativity of spastin. Several lines of evidence show that two neighbor subunits interact strongly with each other: (i) the ATP-activated ATPase activity shows a Hill coefficient of ~2; (ii) inactive mutant subunits and (iii) non-hydrolyzable ATP analogs inhibit the activity of spastin in a hyperbolic dependence, characteristic for two interacting species. A quantitative model fits the data well, and strongly disfavors orders of cooperativity higher than 2. These observations are relevant for patients suffering from SPG4-type hereditary spastic paraplegia, and can explain why single amino acid exchanges lead to dominant-negative phenotypes. In severing assays, wildtype spastin is even more sensitive towards the presence of inactive mutants than in enzymatic assays, suggesting a weak coupling of ATPase and severing activity. Together, these observations indicate that each of spastin's six catalytic sites depends on the presence of an active neighbor site, and that ATP hydrolysis in all subunits is required for full severing activity.

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One-Dimensional Diffusion of Tau Protein Guided by the Microtubule Lattice

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Current models for the intracellular transport of Tau protein suggest motor protein dependent co-transport with short microtubule fragments and diffusion of Tau in the cytoplasm. In these models Tau is believed to be stationary bound to microtubules and in equilibrium with free diffusion in the cytosol. Recent observations that some members of the microtubule-dependent kinesin family show Brownian motion along MTs led us to hypothesize that diffusion along MTs could also be relevant in the case of Tau. We used single molecule TIRF microscopy to probe for diffusion of individual fluorescently labelled Tau molecules along immobilized microtubules. This allowed us to circumvent masking of microtubules dependent diffusion of Tau by excess of labelled Tau protein in solution that might occur in in vivo overexpression experiments. We found that about half of the individually detected Tau molecules were able to diffuse bi-directionally along microtubules in the absence of ATP. Diffusion parameters such as diffusion coefficient, interaction time, and scanned microtubule length did not change with Tau concentration. Tau binding and diffusion of Tau along the microtubule lattice were sensitive to ionic strength and drastically reduced upon enzymatic removal of the negatively charged carboxy termini of tubulin. We propose one-dimensional Tau diffusion guided by the microtubule lattice as one possible additional mechanism for the distribution of Tau. By such one-dimensional microtubule lattice diffusion instead of or in addition to directed motor-dependent transport, both ends of the microtubules, i.e. the sites where Tau protein is needed during microtubule polymerization, could be reached even in situations when Tau levels are pathologically high and active transport along microtubules might be compromised.

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Biophysical Study of Native Yeast Kinetochore Indicates Distinct Roles for Phospho-Regulation of Core Microtubule-Binding Subcomplexes

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Chromosome segregation is orchestrated by multi-protein complexes called kinetochores, which initially bind microtubules and later form persistent load-bearing tip attachments. Accurate segregation requires regulation of kinetochores by Aurora B kinase. One popular view is that Aurora acts on kinetochores that have already established erroneous attachments to microtubule tips, phosphorylating two major microtubule-binding subcomplexes, Ndc80 and Dam1, and thereby triggering detachment. It is unknown whether phosphorylation affects initial binding of kinetochores to microtubules. Moreover, the relative importance of phosphoregulation of these subcomplexes in the context of whole kinetochores is unclear. To address these